

#5

4-10-90

PATENTS
Case 21060

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re U.S. Patent No. 4,234,571 :

of John J. Nestor et al. :

Issued: November 18, 1980

To: Syntex (U.S.A) Inc.

Serial No. 47,661 :

Filed June 11, 1979 :

For NONAPEPTIDE AND DECAPEPTIDE DERIVATIVES OF LUTEINIZING
HORMONE RELEASING HORMONE

Honorable Commissioner of Patents and Trademarks
Box Patent Ext.
Washington, D.C. 20231

Sir:

APPLICATION FOR EXTENSION OF PATENT TERM
UNDER 35 U.S.C. 156 and 37 C.F.R. 1.710 et seq.

This document is a request for a two-year extension of the term of the patent covering nafarelin acetate, the active component in SYNAREL® standard nasal spray solution. The patent number is U.S. Patent 4,234,571 issued November 18, 1980 and is referred to in this document as "the Patent."

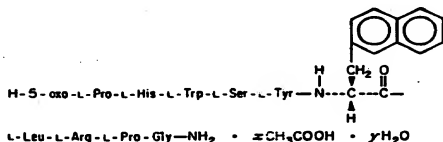
Applicant represents that it is the assignee of the entire interest in and to U.S. Patent No. 4,234,571, issued in the names of John J. Nestor, Gordon H. Jones and Brian H. Vickery, by virtue of the assignment recorded on November 13, 1979, at Reel 3701, Frame 237-239, a true copy of which is hereto attached as "Attachment A."

Applicant submits this Application For Extension Of Patent Term for U.S. Patent 4,234,571 by providing the following information, as required by 35 U.S.C 156 and 37 C.F.R. 1.710

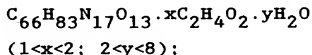
et. seq. For the convenience of the Patent and Trademark Office, the information contained in this application will be organized as is set out in 37 C.F.R. 1.740(a).

(1) The approved product is identified as SYNAREL[®] standard nasal solution. It comprises a compound having:

(a) the following structural formula:



(b) the molecular formula:



(c) a molecular weight of 1322.51 (anhydrous, free base form);

(d) the chemical names (i) Luteinizing hormone-releasing factor (or hormone)(pig), 6-[3-(2-naphthalenyl)-D-alanine]-, acetate (salt), hydrate; and (ii) 5-oxo-L-prolyl-L-histidyl-L-tryptophyl- L-seryl-L-tyrosyl-3-(2-naphthyl)-D-alanyl-L-leucyl-L- arginyl-L-pyroglycinamide acetate (salt) hydrate (as set forth in the 1990 USAN and the USP Dictionary of Drug Names at page 379);

(e) the generic name: nafarelin acetate (salt), hydrate and nafarelin; and

(f) the CAS registry numbers (i) CAS-86220-42-0 (nafarelin acetate (salt) hydrate); and (ii) CAS-76932-56-4 (nafarelin).

(2) One dosage strength of SYNAREL® standard nasal solution (dosage form 2.0 mg/ml) for administration of the approved product was subject to regulatory review under Section 505(b) of the Federal Food, Drug and Cosmetic Act.

(3) SYNAREL® standard nasal solution received permission for commercial marketing under Section 505(b) of the Federal Food, Drug and Cosmetic Act on February 13, 1990.

(4) SYNAREL® standard nasal solution contains as the sole active ingredient nafarelin acetate (salt), hydrate, described above in item (1). This product has not been previously approved for commercial marketing under Section 505(b) of the Food, Drug and Cosmetic Act.

(5) This Application for extension of the patent term under 35 U.S.C. 156 is being submitted within the permitted 60 day period; said period will expire on April 13, 1990.

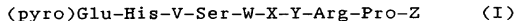
(6) The patent for which patent term extension is sought is U.S. Patent No. 4,234,571, which issued on November 18, 1980 on U.S. Serial No. 47,661, filed June 11, 1979, naming John J. Nestor et al. the inventors for NONAPEPTIDE AND DECAPEPTIDE DERIVATIVES OF LUTEINIZING HORMONE RELEASING HORMONE. The term of the patent has never been extended and has not yet expired. The patent will expire on November 18, 1997. No other patent's term has been extended for the same regulatory review period for any product containing nafarelin acetate (salt), hydrate.

(7) A complete copy of the U.S. Patent No. 4,234,571 in the prescribed form is hereto attached as "Attachment B."

(8) There has been no disclaimer, certificate of correction, receipt of maintenance fee payments or reexamination certificate issued with regard to U.S. Patent No. 4,234,571.

(9) U.S. Patent No. 4,234,571 claims the approved product in the following applicable claims:

Claim 1 covers, inter alia, nafarelin acetate, that is, a compound represented by the formula



wherein V is Trp; W is Tyr; X is 3-(-2-naphthyl)-D-alanyl; Y is Leu; and Z is Gly-NH₂ and its pharmaceutically acceptable salts.

Claim 2: same analysis as Claim 1; and

Claim 3: same analysis as Claim 1.

Claim 12 covers, inter alia, a composition for treating endometriosis in a female comprising an effective amount of a compound of the formula (I) above.

Claim 13 covers, inter alia, a method for treating endometriosis in a female which comprises administering to said subject an effective amount of a compound of the formula in (I) above.

(10) The relevant dates and information pursuant to 35 U.S.C. 156(g) are as follows:

For SYNAREL[®] standard nasal solution:

- (a) November 19, 1980: IND 18,138 received by FDA
- (b) May 18, 1981: Revised protocol for ICM 808 to
IND 18,138 submitted; and
effective date of IND 18,138
- (c) November 22, 1988: NDA 19-886 received by FDA
- (d) February 13, 1990: NDA 19-886 approved by FDA

(11) The following is a brief description of the activities undertaken by applicant during the applicable regulatory review period:

November 5, 1980	Syntex submits IND 18,138 New Study ICM 808 submitted with original IND.
November 19, 1980	FDA acknowledges receipt of IND 18,138.
May 18, 1981	Syntex submits revised protocol for ICM 808 to IND 18,138. Effective date of IND 18,138.
November 5, 1981	Syntex submits amendment to IND 18,138 advising FDA that anniversary date for progress reports is May 18, 1981.
January 26, 1982	Syntex submits final reports of preclinical teratology to IND 18,138.
February 22, 1982	Syntex submits new study ICM 906 to IND 18,138.
March 31, 1982	Syntex submits pre-meeting information for pharmaceutical development program to IND 18,138.
May 6, 1982	Syntex submits new study ICM 892 to IND 18,138.
May 19, 1982	Syntex submits first annual report to IND 18,138.

July 23, 1982	Syntex submits new study ICM 909 to IND 18,138.
August 6, 1982	Syntex submits new study ICM 969 to IND 18,138.
October 21, 1982	Syntex submits new studies ICM 939 and ICM 885 to IND 18,138.
December 22, 1982	Syntex submits new study ICM 1010 and a final report for a toxicity study to IND 18,138.
January 12, 1983	Syntex submits pre-meeting information for formulation development program.
May 19, 1983	Syntex submits second annual report to IND 18,138.
May 24, 1983	Syntex submits documentation of agreements reached in meeting of January 26, 1983 regarding development program for nafarelin acetate.
September 21, 1983	Syntex submits final report of preclinical pharmacology/toxicology to IND 18,138.
October 7, 1983	Syntex submits new study ICM 951 to IND 18,138.
November 15, 1983	Syntex submits third annual report to IND 18,138 and re-establishes report date.

February 10, 1984 Syntex submits new study ICM 1042 to IND
18,138.

March 2, 1984 Syntex submits data on potential teratologic
effects of LHRH analogs in subhuman primates
to IND 18,138.

March 20, 1984 Syntex submits new study ICM 1067 to IND
18,138.

May 3, 1984 Syntex submits agenda for meeting to discuss
issues on FRS allowability and testing.

May 10, 1984 Syntex submits new study ICM 1159 to IND
18,138.

October 25, 1984 Syntex submits new study ICM 1196 and ICM 1216
to IND 18,138. Syntex submits fourth annual
report to IND 18,138. Syntex consolidates IND
18,131 and 18,138 - all future filings will be
made to IND 18,138.

November 27, 1984 Syntex submits new study ICM 1223 to IND
18,138.

March 6, 1985 Syntex submits new study ICM 1041 to IND
18,138.

March 28, 1985 Syntex submits pre-meeting information to
discuss development program for nafarelin
acetate.

June 4, 1985 Syntex submits new study ICM 1247 to IND
18,138.

July 30, 1985 Syntex submits new study ICM 1248 to IND
18,138.

September 11, 1985 Syntex requests meeting with FDA to discuss
adequacy of one large multicenter study to
support NDA approval.

November 12, 1985 Syntex requests meeting with FDA to discuss
chemistry/pharmacy issues for the NDA filing.

 Syntex submits final protocols to be used in
evaluating toxicity of nafarelin nasal
solutions.

November 15, 1985 Syntex submits new study ICM 1287 to IND
18,138.

December 20, 1985 Syntex submits fifth annual report to IND
18,138

January 24, 1986 Syntex submits pre-meeting information to
discuss the adequacy of the endometriosis and
prostate carcinoma clinical development
programs.

March 12, 1986	Syntex submits comments regarding the adequacy of the endometriosis and prostatic carcinoma clinical development programs to support NDA filing to IND 18,138.
March 14, 1986	Syntex submits pre-meeting information for meeting with FDA to discuss Nafarelin formulation development.
April 1, 1986	Syntex submits information to clarify clinical development program to IND 18,138.
April 4, 1986	Syntex submits new study ICM 1339 and ICM 1330 to IND 18,138.
April 29, 1986	Syntex submits new study ICM 1342 to IND 18,138.
June 2, 1986	Syntex combines ongoing studies ICM 1123 and ICM 1163 as new study ICM 1123A all under IND 18, 138. Syntex submits new study ICM 1123B to IND 18,138.
July 3, 1986	Syntex submits new study Labs 25-6280 to IND 18,138.

July 24, 1986	Syntex submits summary of preliminary carcinogenicity findings to IND 18,138.
	Syntex submits response on FDA letter regarding pharmacokinetic/bioavailability studies to IND 18,138.
August 6, 1986	Syntex submits amendment to modify synthesis of nafarelin acetate to IND 18,138.
August 29, 1986	Syntex submits new study ICM 1383 to IND 18,138.
November 3, 1986	Syntex submits sixth annual report to IND 18,138.
April 2, 1987	Syntex submits new protocol ICM 1412 to IND 18,138.
May 29, 1987	Syntex submits new study ICM 1370 and ICM 1395 to IND 18,138.
June 5, 1987	Syntex submits pharmacology/toxicology final reports to IND 18,138.
October 6, 1987	Syntex submits pre-meeting information on NDA organization and clinical data analysis and presentation to IND 18,138.

November 20, 1987 Syntex submits pharmacology/toxicology final reports to IND 18,138.

December 8, 1987 Syntex submits seventh annual report to IND 18,138.

January 29, 1988 Syntex submits requested information to IND 18,138 from pre-NDA meeting on October 27, 1987 regarding power calculations to be used in pivotal endometriosis clinical trials.

February 3, 1988 Syntex submits pharmacology/toxicology final reports to IND 18,138.

April 6, 1988 Syntex submits new study ICM 1557 to IND 18,138.

April 13, 1988 Syntex submits pharmacology/toxicology final reports to IND 18,138.

June 13, 1988 Syntex submits new study ICM 1468 to IND 18,138. Syntex submits application for orphan drug designation for nafarelin acetate for the treatment of central precocious puberty.

June 22, 1988 FDA acknowledged receipt of Syntex submissions dated June 13, 1989.

June 30, 1988 Syntex submits an update to FDA on the status of activities relating to the filing of the NDA for nafarelin acetate for endometriosis.

 Syntex submits new protocol LAB/NAFn031/USA to IND 18,138.

July 20, 1988 FDA letter grants orphan drug designation for Nafarelin acetate for treatment of central precocious puberty.

September 9, 1988 Syntex submits new protocol ICM 1467 to IND 18,138.

October 14, 1988 Syntex submits pharmacology/toxicology final report to IND 18,138. Syntex submits new protocol ICS/NAFn043/USA.

November 1, 1988 Syntex submits new protocol ICM 1628 to IND 18,138.

November 8, 1988 Syntex submits clinical pharmacology final report and internal report on steroids to IND 18,138.

November 11, 1988 Syntex submits the NDA for Synarel (nafarelin acetate) Nasal Solution to FDA.

November 22, 1988 FDA acknowledged receipt of NDA for Synarel (nafarelin acetate) Nasal Solution and assigns NDA number 19-886 to submission.

February 6, 1989 Syntex submits copies of published information relating to Syntex clinical experience in treating endometriosis to NDA 19-886.

Syntex submits letter confirming meeting to discuss agenda for Maternal Health and Fertility Advisory Committee.

February 24, 1989 Syntex submits documents for trials identified by Syntex as adequate and well-controlled to IND 18,138 and to NDA 19-886.

March 10, 1989 Syntex submits a request to FDA for changes in the proposed agenda for the Maternal Health and Fertility Advisory Committee.

March 13, 1989 Syntex submits information in support of the statistical review of mouse and rat carcinogenicity studies to NDA 19-886.

March 23, 1989 Syntex submits background documents to FDA for the April 28, 1989 meeting of the Fertility and Maternal Health Drugs Advisory Committee.

March 27, 1989 Syntex submits new study ICM 1676 to IND 18,138.

March 29, 1989 Syntex submits response to request for reanalysis of clinical data to NDA 19-886.

March 31, 1989 Syntex submits response to request for calculation of 95% 2-sided confidence intervals.

April 4, 1989 Syntex submits tables and figures to be used by Syntex speakers at the April 28, 1989 Maternal Health and Fertility Advisory Committee Meeting to NDA 19-886.

April 7, 1989 Syntex submits an update of safety information to NDA 19-886.

April 17, 1989 Syntex submits a report to NDA 19-886.

April 24, 1989 Syntex submits eighth annual report to IND 18,138.

May 31, 1989 Syntex submits response to FDA's request regarding bone density to NDA 19-886.

June 5, 1989 Syntex submits draft labeling for Synarel (nafarelin acetate) to NDA 19-886.

June 6, 1989 Syntex submits word processing disks of the revised Synarel (nafarelin acetate) labeling to NDA 19-886.

June 12, 1989 Syntex submits draft Summary Basis of Approval for Synarel (nafarelin acetate) to NDA 19-886.

June 15, 1989	Syntex submits word processing disks of the Summary Basis of Approval for Synarel (nafarelin acetate) to NDA 19-886.
July 6, 1989	Syntex submits revised draft labeling to NDA 19-886.
July 17, 1989	Syntex submits revised draft labeling to NDA 19-886.
August 9, 1989	Syntex submits revised draft labeling to NDA 19-886.
August 11, 1989	Syntex submits new protocol LAB/NAFn601/USA to IND 18,138
August 22, 1989	Syntex submits revised draft labeling to NDA 19-886.
September 6, 1989	Syntex submits revised draft labeling to NDA 19-886.
September 14, 1989	Syntex submits revised draft labeling to NDA 19-886.
September 15, 1989	Syntex submits revised draft labeling to NDA 19-886.

September 20, 1989 Syntex submits new protocol ICS/NAFn051/USA to IND 18,138. Syntex submits pharmacology/toxicology final reports to IND 18,138.

November 6, 1989 Syntex submits information to NDA 19-886 for biopharmaceutics review.

November 8, 1989 Syntex submits revised draft labeling to NDA 19-886.

November 14, 1989 Syntex submits revised draft labeling to NDA 19-886.

November 27, 1989 Syntex submits an update of Safety Information to NDA 19-886.

November 28, 1989 Syntex submits revised draft labeling to NDA 19-886.

December 1, 1989 Syntex submits the introductory advertising campaign to NDA 19-886.

December 5, 1989 Syntex submits samples for methods validation to reviewing chemists.

December 7, 1989 Syntex submits revised draft labeling to NDA 19-886 in response to FDA's Medical, Chemistry and Biopharmaceutics review.

December 8, 1989 Syntex submits the ninth annual report to IND 18,138.

December 20, 1989 Syntex requests agenda assignments to present the Nafarelin precocious puberty program at the March Advisory Committee Meeting.

January 5, 1990 Syntex submits information regarding the March Advisory Committee Meeting.

February 7, 1990 Syntex submits revised nafarelin package insert dated 2/07/90 to NDA 19-886.

February 9, 1990 Syntex submits final revised nafarelin package insert dated 2/09/90 to NDA 19-886. Letter submitted on nafarelin dose ranging to NDA 19-886.

February 14, 1990 Approval letter on NDA 19-886, dated February 13, 1990, received from FDA via telefax.

(12) In the opinion of Applicant, the patent is eligible for the requested extension.

The regulatory review period started on May 18, 1981 the day that the IND 18,138 became effective under subsection (i) of Section 505 of the U.S. Food, Drug and Cosmetic Act. This was subsequent to the issuance of U.S. Patent No. 4,234,571 on November 18, 1980.

The regulatory review period lasted 8 years, 8 months, and 26 days (3,191 days, including 2 leap years) until February 13, 1990.

The period from the effective date of the IND (18,138) to the effective date of receipt by the FDA of the NDA (19-886) on November 22, 1988 is 7 years and 189 days (or 2744 days, including 1 leap year); half of this period is 1372 days.

The period from the date of receipt of NDA 19-886 on November 22, 1988 to the date of NDA approval on February 13, 1990 is 1 year, 2 months and 21 days (448 days).

The total sum of the possible extension is 1,820 days as calculated by the following method:

1372 days + 448 days = 1820 days or 4.99 years

However, 35 U.S.C. 156(g)(4)(C) applies because U.S. Patent No. 4,234,571 issued before the date of enactment of 35 U.S.C. 156, the IND was filed before enactment, and the product was approved after enactment. Therefore, Applicant requests a two-year extension of the terms of U.S. Patent No. 4,234,571.

The total term of the patent remaining after the date of approval of the approved product, including the two-year extension, is 6 years, 9 months and 23 days, which does not exceed the maximum 14-year term pursuant to 35 U.S.C. 156(c)(3).

(13) Applicant acknowledges the duty to disclose to the Commissioner of Patent and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the extension sought in this Application For Extension Of Patent Term.

(14) Charge the fee of \$550.00 due under 37 CFR 1.20(n) to Deposit Account No. 19-5430.

(15) Inquiries and correspondence relating to this Application for Extension Of Patent Term are to be directed to:

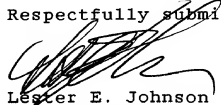
Tom M. Moran, Esq.
Syntex (U.S.A.) Inc.
3401 Hillview Avenue
P.O. Box 10850
Palo Alto, CA 94303
(415)855-6137

(16) This Application for Extension Of Patent Term is being submitted in duplicate, certified as such below.

(17) The undersigned hereby declares that he is a patent attorney authorized to practice before the United States Patent and Trademark Office and has general authority from Applicant, Syntex (U.S.A.) Inc., the owner of the patent, for the purpose of transacting all matters reasonably related to obtaining an extension of patent term for U.S. Patent No. 4,234,571, to act on its behalf in patent matters; that he has reviewed and understands the contents of the application being submitted pursuant to 35 U.S.C. 156; that he believes the patent is subject to extension pursuant to 37 CFR 1.710; that the

believes an extension of the length claimed is fully justified under 35 U.S.C. 156, and the applicable regulations; and that he believes the patent for which the extension is being sought meets the conditions for extension of the term of a patent as set forth in 37 CFR 1.720.

Respectfully submitted,

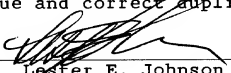


Lester E. Johnson
Attorney for Applicants
Reg. No. 28,023

Syntex (U.S.A.) Inc.
3401 Hillview Avenue
P.O. Box 10850
Palo Alto, California 94303
(415)855-6593
April 05, 1990

LEJ:3954M

This is to certify that the copy of this Application (together with the appended Attachments A and B) filed herewith is a true and correct duplicate.



Lester E. Johnson

April 06, 1990
Date

ASSIGNMENT

WHEREAS, we, JOHN J. NESTOR, GORDON H. JONES and BRIAN H. VICKERY of San Jose, California; Cupertino, California; and Cupertino, California, respectively, have invented certain new and useful improvements in NONAPEPTIDE AND DECAPEPTIDE DERIVATIVES OF LUTEINIZING HORMONE RELEASING HORMONE

which is described in an application for Letters Patent of the United States executed by us on June 8, 1979 and filed in the United States Patent Office on June 11, 1979 under Serial No. 47,661;

AND WHEREAS, SYNTEX (U.S.A.) INC., a corporation of Delaware having an address at 3401 Hillview Avenue, Palo Alto, California 94304, is desirous of acquiring an interest therein and in the Letters Patent to be obtained therefor from the United States;

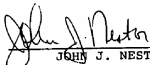
NOW, THEREFORE, be it known by all whom it may concern, that for and in consideration of One Dollar (\$1.00) and other good and valuable considerations, the receipt of which is hereby acknowledged, we, JOHN J. NESTOR, GORDON H. JONES and BRIAN H. VICKERY, by these presents do hereby

assign, sell and transfer unto the said SYNTEX (U.S.A.) INC., for the territory of The United States of America and for all foreign countries, the full and exclusive right, title and interest, including all rights under the Paris Convention for the Protection of Industrial Property, in and to said invention, as fully set forth and described in the specification of the application for Letters Patent of the United States executed by us; said invention, application and Letters Patent to be held and enjoyed by the said

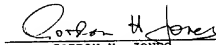
SYNTEX (U.S.A.) INC., for its own use and behoof, and for the use and behoof of its successors, assigns and legal representatives, to the full end of the term for which said Letters Patent may be granted as fully and entirely as the same would have been held by us had this assignment and sale not been made.

IN WITNESS WHEREOF, we, JOHN J. NESTOR, GORDON H. JONES and BRIAN H. VICKERY have hereunto set our hands and affixed our seals to this assignment.

11/6/79
(Date)

 (SEAL)
JOHN J. NESTOR

11/6/79
(Date)

 (SEAL)
GORDON H. JONES

11/6/79
(Date)

 (SEAL)
BRIAN H. VICKERY

NEEL3701 FRAME238

United States Patent [19]

Nestor et al.

[11] 4,234,571

[45] Nov. 18, 1980

- [54] **NONAPEPTIDE AND DECAPEPTIDE DERIVATIVES OF LUTEINIZING HORMONE RELEASING HORMONE**
- [75] Inventors: John J. Nestor, San Jose; Gordon H. Jones; Brian H. Vickery, both of Cupertino, all of Calif.
- [73] Assignee: Syntex (U.S.A.) Inc., Palo Alto, Calif.
- [21] Appl. No.: 47,661
- [22] Filed: Jun. 11, 1979
- [51] Int. Cl.³ A61K 37/00; C07C 103/52
- [52] U.S. Cl. 424/177; 260/112.5 LH
- [58] Field of Search 260/112.5 LH; 424/177
- [56] **References Cited**

U.S. PATENT DOCUMENTS

3,992,530	11/1976	Foell et al.	260/112.5 LH
4,005,194	1/1977	Johnson	424/177
4,010,125	3/1977	Schally et al.	260/112.5 LH
4,018,726	4/1977	Schally et al.	260/112.5 LH
4,086,219	4/1978	Wittle et al.	260/112.5 LH
4,089,946	5/1978	Foell et al.	260/112.5 LH
4,143,133	3/1979	Foell et al.	260/112.5 LH

Primary Examiner—Delbert R. Phillips
 Attorney, Agent, or Firm—Alan M. Krubiner; Tom M. Moran

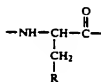
- [57] **ABSTRACT**
- Nonapeptide and decapeptide analogs of LH-RH of the formula



(I)

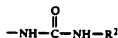
and the pharmaceutically acceptable salts thereof wherein:

V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;
 W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;
 X is a D-amino acid residue



wherein R is

- (a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or
- (b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;
- Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;
 Z is glycineamide or —NH—R¹, wherein
 R¹ is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein
 R² is hydrogen or lower alkyl,
 are disclosed. These compounds exhibit potent LH-RH agonist properties.

15 Claims, No Drawings

NONAPEPTIDE AND DECAPEPTIDE DERIVATIVES OF LUTEINIZING HORMONE RELEASING HORMONE

BACKGROUND OF THE INVENTION

Luteinizing hormone (LH) and follicular stimulating hormone (FSH) are released from the anterior pituitary gland under the control of the releasing hormone LH-RH produced in the hypothalamic region. LH and FSH act on the gonads to stimulate the synthesis of steroid hormones and to stimulate gamete maturation. The pulsatile release of LH-RH, and thereby the release of LH and FSH, controls the reproductive cycle in domestic animals and humans. Additionally, LH-RH has effects in placenta, in releasing HCG, and directly on the gonads. Agonist analogs of LH-RH are useful for the control of fertility by two mechanisms of action. Low doses of LH-RH analogs can stimulate ovulation and are useful in the treatment of hypothalamic and ovulatory infertility. Additionally they can be used for hypogonadal conditions and impotence, and stimulate spermatogenesis and androgen production in the male. Paradoxically, larger doses of highly potent and long-lasting analogues of LH-RH have an opposite effect and block ovulation in the female and suppress spermatogenesis in the male. Related to these effects is a suppression of normal circulating levels of sexual steroids of gonadal origin, including reduction in accessory organ weight in the male and the female. In domestic animals this paradoxical effect promotes weight gain in a feedlot situation, stimulates abortion in pregnant animals and in general, acts as a chemical sterilant.

The natural hormone releasing hormone LH-RH is a decapeptide comprised of naturally occurring amino acid (which have the L-configuration except for the archinal amino acid glycine). Its sequence is as follows: (pyro) Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH₂. Many analogues of this natural material have been studied and the very large majority of them have proven to be of insufficient biological activity to be clinically useful. Certain select modifications have proven to have a beneficial effect on biological activity. By far the most significant modification is obtained by changing the 6-position residue from Gly to a D-amino acid. For example, replacing the Gly residue in the 6-position by D-Ala, D-Leu, D-Phe or D-Trp has led to a series of analogues of LH-RH with increased activity relative to LH-RH. See M. Monahan, et al., *Biochem.*, 12, 4616 (1973) for [D-Ala⁶]-LHRH; J. A. Vilchez-Martinez, et al., *Biochem. Biophys. Res. Comm.*, 59, 1226 (1974) for [D-Leu⁶]-LHRH and desGly¹⁰[D-Leu⁶, Pro NHE⁹]-LHRH; D. H. Coy, et al., *J. Med. Chem.*, 19, 423 (1976) for [D-Phe]-LHRH; and W. Vale, et al., *Clinical Endocrinology*, 5th Supp., Blackwell Scientific Publications, Oxford, England (1976), p. 2615 and D. H. Coy, et al., *Biochem. Biophys. Res. Comm.*, 67, 576 (1979) for [D-Trp⁶]-LHRH.

In addition to the substantial increases in activity obtained by the above-referred to substitutions in position 6, further increases in activity may be obtained by eliminating the Gly-NH₂ in position 10 to afford a nonapeptide as an alkyl-, cycloalkyl- or fluoroalkylamide, or by replacing Gly-NH₂ by an α-azaglycine amide. See for example, M. Fujino, et al., *Biochem. Biophys. Res. Comm.*, 49, 863 (1972), D. H. Coy, et al., *Biochem.*, 14,

1848(1975) and A. S. Dutta, et al., *J. Chem. Soc. Perkin I*, 1979, 379.

Substitution of N-methyl-leucine for the leucine residue in position 7 leads to increased stability towards enzymatic degradation. See for example, N. Ling, et al., *Biochem. Biophys. Res. Comm.*, 63, 801 (1975).

Substitution of the tryptophan residue in position 3 by 3-(1-naphthyl)-L-alanine leads to an increase in biological potency. See for example, K.U. Prasad, et al., *J. Med. Chem.*, 19, 492 (1976) and Y. Yabe, *Chem. Pharm. Bull.*, 24 (12), 3149 (1976).

The tyrosine residue in position 5 can be replaced by phenylalanine or 3-(1-pentafluorophenyl)-L-alanine with the retention of substantial biological activity. See for example, N. Yanaihara, et al., *Biochem. Biophys. Res. Comm.*, 52, 64 (1973), and D. Coy, et al., *J. Med. Chem.*, 16, 877 (1973).

It would be desirable to prepare further analogues of LH-RH which have even a higher degree of biological activity than those heretofore described and which can be used clinically in animals and humans.

SUMMARY OF THE INVENTION

The present invention refers to novel nonapeptide and decapeptide derivatives of LH-RH which have, in the 6-position, certain lipophilic D-amino acids. The invention is also directed to various methods of use of these compounds and to pharmaceutical compositions thereof. A further aspect of the invention involves processes for the preparation of the novel compounds described above and to intermediates useful in such processes.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to novel nonapeptide and decapeptide derivatives of LH-RH. More particularly the present invention relates to derivatives of LH-RH which have, in the 6-position, specific unnatural D-amino acid residues containing lipophilic carbocyclic residues, particularly residues containing two or more carbocyclic aryl (or perhydroaryl) rings or a phenyl (or cyclohexyl) ring which is highly alkyl substituted.

More specifically the compounds of the present invention are nonapeptides and decapeptides of the formula

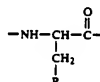


and the pharmaceutically acceptable salts thereof wherein:

V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

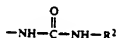
X is a D-amino acid residue



wherein R is

(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl,

- phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or
- (b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydro-naphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;
- Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl; Z is glycylamide or -NH-R¹, wherein R¹ is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R² is hydrogen or lower alkyl.

As set forth above and for convenience in describing this invention, the conventional abbreviations for the various common amino acids are used as generally accepted in the peptide art as recommended by the IUPAC-IUB Commission on Biochemical Nomenclature, *Biochemistry*, 11, 1726 (1972) and represent L-amino acids with the exception of the achiral amino acid glycine and with the further exception of the amino acids in the 6-position designated by X. All peptide sequences mentioned herein are written according to the generally accepted convention whereby the N-terminal amino acid is on the left and the C-terminal amino acid is on the right.

As used herein, the term "pharmaceutically acceptable salts" refer to salts that retain the desired biological activity of the parent compound and do not impart any undesired toxicological effects. Examples of such salts are (a) acid addition salts formed with inorganic acids, for example hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid and the like; and salts formed with organic acids such as, for example, acetic acid, oxalic acid, tartaric acid, succinic acid, maleic acid, fumaric acid, gluconic acid, citric acid, malic acid, ascorbic acid, benzoic acid, tannic acid, pantoic acid, alginic acid, polyglutamic acid, naphthalenesulfonic acids, naphthalenedisulfonic acids, polygalacturonic acid; (b) salts with polyvalent metal cations such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium, and the like; or with an organic cation formed from N, N'-dibenzylethylenediamine or ethylenediamine; or (c) combinations, of (a) and (b), e.g. a zinc tannate salt and the like.

As used herein the term "lower alkyl" refers to a straight or branched chain saturated hydrocarbon group having from 1 to 4 carbon atoms such as, for example, methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, sec-butyl and tert-butyl; the term "cycloalkyl group" refers to a cyclic saturated hydrocarbon group having from 3 to 6 carbon atoms, for example cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl; the term "fluoro lower alkyl" refers to a lower alkyl group wherein one or more hydrogen atoms are replaced by fluorine, such as, for example, trifluoromethyl, pentafluoroethyl, 2,2,2-trifluoroethyl, and the like.

As used herein "naphthyl" is inclusive of 1- and 2-naphthyl; "anthryl" is inclusive of 1-, 2- and 9-anthryl; "fluorenyl" is inclusive of 2-, 3-, 4- and 9-fluorenyl; "phenanthryl" is inclusive of 2-, 3-, and 9-phenanthryl; and "adamantyl" is inclusive of 1- and 2-adamantyl.

Preferred compounds of this invention are those wherein X is 3-(2-naphthyl)-D-alanyl or 3-(2,4,6-trimethylphenyl)-D-alanyl; Z is glycylamide or -NHEt; V is tryptophyl or phenylalanyl; W is tyrosyl and Y is leucyl or N-methyl-leucyl. Particularly preferred compounds are

- (pyro) Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂;
- (pyro) Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-N-methyl-Leu-Arg-Pro-Gly-NH₂;
- (pyro)Glu-His-Phe-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂;
- (pyro) Glu-His-Trp-Ser-Tyr-3-(2,4,6-trimethylphenyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂;
- (pyro) Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-NHEt; and
- (pyro) Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-N-methyl-Leu-Arg-Pro-NHEt; and their pharmaceutically acceptable salts.

Especially preferred is (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂ and its salts.

The compounds of this invention and, particularly, the salts thereof, exhibit surprisingly potent and long lasting LH-RH agonist activity in comparison to the previously most potent LH-RH agonists, namely (pyro)Glu-His-Trp-Ser-Tyr-D-Trp-Ser-Arg-Pro-Gly-NH₂ and the corresponding prolylethylamide. A primary measure of potency is the ability to partially or completely suppress estrus in normally cycling adult female rats (determined over a 2 week period) by twice daily subcutaneous injection.

Other bioassays which have been used for LH-RH analogues and which have been used for compounds of the present invention include:

(a) ovulation induction in diestrous or proestrous female rats by subcutaneous injection (Rippel, et al. *Proc. Soc. Exp. Biol. Med.*, 148, 1193(1975)),

(b) LH and FSH release by dispersed anterior pituitary cell cultures as measured by radioimmunoassay (Vale, et al. *Endocrinology*, 91, 562(1972)), and

(c) LH and FSH release into the peripheral circulation of ovariectomized, steroid treated rats in response to intravenous injection as measured by radioimmunoassay (Arimura, et al. *Endocrinology*, 90, 163(1972)).

On a more advanced level, activity for these compounds may be demonstrated in vivo by depression of spermatogenesis and circulating and testicular levels of testosterone as well as dramatic reduction in prostate size in dogs suffering from benign prostatic hypertrophy.

As a result of the above the compounds may find use in a large variety of situations where control of LH and FSH, or direct gonadal action is important, including:

PHYSIOLOGICAL UTILITIES (LOW DOSE EFFECTS)

ovulation induction in anovulatory infertility and for timed ovulation in female mammals;

therapy for infertility due to insufficient luteal function in women;

therapy for hypogonadotropic or hypogonadal infertility in either sex-human.

therapy for cystic ovary/nymphomania syndrome in cattle;

induction or enhancement of sexual behaviour or therapy for impotence/frigidity.

PARADOXICAL UTILITIES (HIGH DOSE EFFECTS)

female contraception;

ovulation suppression or delay;

induction of parturition;

synchronization of ovulation;

estrus suppression;

growth promotion in female animals;

luteolysis, menses induction;

early, first trimester abortifacient;

therapy for endometriosis;

therapy for mammary tumors and cysts therapy for

polycystic ovary syndrome (Stein-Leventhal);

therapy for uterine carcinoma;

therapy for benign prostatic hypertrophy and for

prostatic carcinoma;

male contraception;

therapy for diseases which result from excessive gon-

adal hormone production in either sex;

functional castration in male food producing animals;

suppression of proestrous discharge.

Another aspect of the present invention relates to

particular uses for the above-described compounds,

(including uses not heretofore described for LH-RH

analogues) namely their uses for inhibiting ovulation

(i.e. contraception) in the female, in the management of

endometriosis, in the treatment of benign prostatic hyper-

trophy and in the inhibition of spermatogenesis (i.e.

contraception) in the male. Thus, in these aspects, the

invention is directed to a method useful for inhibition of

ovulation, management of endometriosis, reduction of

prostate size or inhibition of spermatogenesis in a mam-

malian subject having need of or desiring, said treat-

ment which comprises administering to said subject an

effective amount of a compound of the present inven-

tion as hereinabove described or a pharmaceutical com-

position containing same.

In the practice of the method of this invention an

effective amount of a compound of the invention or a

pharmaceutical composition containing same is admin-

istered to the subject in need of, or desiring, such treat-

ment. These compounds or compositions may be admin-

istered by any of a variety of routes depending upon

the specific end use, including orally, parenterally (in-

cluding subcutaneous, intramuscular and intravenous

administration), vaginally (particularly for contracep-

tion), rectally, buccally (including sublingually), or

intranasally. The most suitable route in any given case

will depend upon the use, particular active ingredient,

the subject involved, and the judgment of the medical

practitioner. The compound or composition may also

be administered by means of slow-release, depot or

implant formulations as described more fully hereinbe-

low.

In general for the uses hereinabove described, which

are so-called "paradoxical" or high-dose uses, it is ex-

pected to administer the active ingredient in amounts

between about 0.01 and 100 $\mu\text{g/kg}$ body weight per

day, preferably between about 0.1 and 5.0 $\mu\text{g/kg}$ body

weight per day. This administration may be accom-

plished by a single daily administration, by distribution

over several applications or by slow release in order to

achieve the most effective results.

The exact dose and regimen for administration of

these compounds and compositions will necessarily be

dependent upon the needs of the individual subject

being treated, the type of treatment, the degree of afflic-

tion or need and, of course, the judgment of the medical practitioner. In general, parenteral administration requires lower dosage than other methods of administration which are more dependent upon absorption.

A further aspect of the present invention relates to pharmaceutical compositions containing as active ingredient a compound of the present invention which compositions comprise such compound in admixture with a pharmaceutically acceptable, non-toxic carrier. As

mentioned above, such compositions may be prepared for use for parenteral (subcutaneous, intramuscular or intravenous) administration particularly in the form of liquid solutions or suspensions; for use in vaginal or

rectal administration particularly in semisolid forms such as creams and suppositories; for oral or buccal administration particularly in the form of tablets or capsules; or intranasally particularly in the form of

powders, nasal drops or aerosols.

The compositions may conveniently be administered in unit dosage form and may be prepared by any of the methods well-known in the pharmaceutical art, for example as described in Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, PA., 1970. Formulations for parenteral administration may

contain as common excipients sterile water or saline, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. Formulations for vaginal or rectal administration,

e.g. suppositories, may contain as excipients, for example, polyalkyleneglycols, vaseline, cocoa butter, and the like. Formulations for inhalation administration may be

solid and contain as excipients, for example, lactose or may be aqueous or oily solutions for administration in the form of nasal drops. For buccal administration typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinized starch, and the like.

It is particularly desirable to deliver the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year from a single administration. Various slow release, depot or implant dosage forms may be utilized.

For example, a dosage form may contain a pharmaceutically acceptable non-toxic salt of the compound which has a low degree of solubility in body fluids, for example, (a) an acid addition salt with a polybasic acid such as phosphoric acid, sulfuric acid, citric acid, tartaric acid, tannic acid, pamoic acid, alginic acid, poly-

glutamic acid, naphthalene mono- or di-sulfonic acids, polygalacturonic acid, and the like; (b) a salt with a polyvalent metal cation such as zinc, calcium, bismuth, barium, magnesium, aluminum, copper, cobalt, nickel, cadmium and the like, or with an organic cation formed from e.g., N,N'-dibenzylethylenediamine or ethylenedi-

amine; or (c) combinations of (a) and (b) e.g. a zinc tannate salt. Additionally, the compounds of the present invention or, preferably, a relatively insoluble salt such as those just described, may be formulated in a gel, for example, an aluminum monostearate gel with, e.g. sesame oil, suitable for injection. Particularly preferred salts are zinc salts, zinc tannate salts, pamoate salts, and the like. Another type of slow release depot formulation for injection would contain the compound or salt dispersed or encapsulated in a slow degrading, non-toxic, non-anitogenic polymer such as a polylactic acid/polyglycolic acid polymer for example as described in U.S. Pat. No. 3,773,919. The compounds or, preferably, relatively insoluble salts such as those described above may also be formulated in cholesterol matrix silastic pellets,

particularly for use in animals. Additional slow release, depot or implant formulations, e.g. liposomes, are well known in the literature. See, for example, "Sustained and Controlled Release Drug Delivery Systems", J. R. Robinson ed., Marcel Dekker, Inc., New York, 1978. Particular reference with respect to LH-RH type compounds may be found, for example, in U.S. Pat. No. 4,010,125.

The polypeptides of the present invention may be synthesized by any techniques that are known to those skilled in the peptide art. An excellent summary of the many techniques so available may be found in J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman Co., San Francisco, 1969, and J. Meienhofer, "Hormonal Proteins and Peptides", Vol. 2, p. 46, Academic Press (New York), 1973 for solid phase peptide synthesis and E. Schroder and K. Lubke, "The Peptides", Vol. 1, Academic Press (New York), 1965 for classical solution synthesis.

In general, these methods comprise the sequential addition of one or more amino acids or suitably protected amino acids to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid is protected by a suitable protecting group. The protected or derivatized amino acid can then be either attached to an inert solid support or utilized in solution by adding the next amino acid in the sequence having the complementary (amino or carboxyl) group suitably protected, under conditions suitable for forming the amide linkage. The protecting group is then removed from this newly added amino acid residue and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining protecting groups (and any solid support) are removed sequentially or concurrently, to afford the final polypeptide. By simple modification of this general procedure, it is possible to add more than one amino acid at a time to a growing chain, for example, by coupling (under conditions which do not racemize chiral centers) a protected tripeptide with a properly protected dipeptide to form, after deprotection, a pentapeptide.

A particularly preferred method of preparing compounds of the present invention involves solid phase peptide synthesis.

In this particularly preferred method the α -amino function of the amino acids is protected by an acid or base sensitive group. Such protecting groups should have the properties of being stable to the conditions of peptide linkage formation, while being readily removable without destruction of the growing peptide chain or racemization of any of the chiral centers contained therein. Suitable protecting groups are t-butyloxycarbonyl (Boc), benzoyloxycarbonyl (Cbz), biphenylisopropylloxycarbonyl, t-amylloxycarbonyl, isobornyloxycarbonyl, α,α -dimethyl-3,5-dimethoxybenzyloxycarbonyl, o-nitrophenylsulfonyl, 2-cyano-t-butyloxycarbonyl, 9-fluorenylmethyloxycarbonyl and the like, especially t-butyloxycarbonyl (Boc).

Particularly preferred side chain protecting groups are, for arginine: nitro, p-toluenesulfonyl, 4-methoxybenzenesulfonyl, Cbz, Boc and adamantyloxycarbonyl; for tyrosine: benzyl, o-bromobenzyloxycarbonyl, 2,6-dichlorobenzyl, isopropyl, cyclohexyl, cyclopentyl and acetyl; for serine: benzyl and tetrahydropyranyl; for histidine: benzyl, p-toluenesulfonyl and 2,4-dinitrophenyl.

The C-terminal amino acid is attached to a suitable solid support. Suitable solid supports useful for the above synthesis are those materials which are inert to the reagents and reaction conditions of the stepwise condensation-deprotection reactions, as well as being insoluble in the media used. Suitable solid supports are chloromethylpolystyrene-divinylbenzene polymer, hydroxymethyl-polystyrene-divinylbenzene polymer, and the like, especially chloromethyl-polystyrene-1% divinylbenzene polymer. For the special case where the C-terminus of the compound will be glycine, a particularly useful support is the benzhydrylamino-polystyrene-divinylbenzene polymer described by P. Rivaille, et al, *Helv. Chim. Acta*, 54, 2772 (1971). The attachment to the chloromethyl polystyrene-divinylbenzene type of resin is made by means of the reaction of the N^o-protected amino acid, especially the Boc-amino acid, as its cesium, tetramethylammonium, triethylammonium, 4,5-diazabicyclo[5.4.0]undec-5-ene, or similar salt in ethanol, acetonitrile, N,N-dimethylformamide (DMF), and the like, especially the cesium salt in DMF, with the chloromethyl resin at an elevated temperature, for example between about 40° and 60° C., preferably about 50° C., for from about 12 to 48 hours, preferably about 24 hours. The N^o-Boc-amino acid is attached to the benzhydrylamine resin by means of an N,N'-dicyclohexylcarbodiimide (DCC)/1-hydroxybenzotriazole (HBT) mediated coupling for from about 2 to about 24 hours, preferably about 12 hours at a temperature of between about 10° and 50° C., preferably 25° C. in a solvent such as dichloromethane or DMF, preferably dichloromethane. The coupling of successive protected amino acids can be carried out in an automatic polypeptide synthesizer as is well known in the art. The removal of the N^o-protecting groups may be performed in the presence of, for example, a solution of trifluoroacetic acid in methylene chloride, hydrogen chloride in dioxane, hydrogen chloride in acetic acid, or other strong acid solution, preferably 50% trifluoroacetic acid in dichloromethane at about ambient temperature. Each protected amino acid is preferably introduced in approximately 2.5 molar excess and the coupling may be carried out in dichloromethane, dichloromethane/DMF mixtures, DMF and the like, especially in methylene chloride at about ambient temperature. The coupling agent is normally DCC in dichloromethane but may be N,N'-di-isopropylcarbodiimide or other carbodiimide either alone or in the presence of HBT. N-hydroxysuccinimide, other N-hydroxyimides or oximes. Alternatively, protected amino acid active esters (e.g. p-nitrophenyl, pentafluorophenyl and the like) or symmetrical anhydrides may be used.

At the end of the solid phase synthesis the fully protected polypeptide is removed from the resin. When the linkage to the resin support is of the benzyl ester type, cleavage is by means of aminolysis with an alkylamine or fluoroalkylamine for peptides with a proline C-terminus, or by aminolysis with, for example, ammonia/methanol or ammonia/ethanol for peptides with a glycine C-terminus at a temperature between about 10° and 50° C., preferably about 25° C., for between about 12 and 24 hours preferably about 18 hours. Alternatively, the peptide may be removed from the resin by transesterification, e.g., with methanol, followed by aminolysis. The protected peptide may be purified at this point by silica gel chromatography. The removal of the (side chain) protecting groups from the polypeptide is performed by treating the aminolysis product with, for

example, anhydrous liquid hydrogen fluoride in the presence of anisole or other carbonium scavenger, treatment with hydrogen fluoride/pyridine complex, treatment with tris(trifluoroacetyl)boron and trifluoroacetic acid, by reduction with hydrogen and palladium on carbon or polyvinylpyrrolidone, or by reduction with sodium in liquid ammonia, preferably with liquid hydrogen fluoride, and anisole at a temperature between about -10° and $+10^{\circ}$ C., preferably about 0° C., for between about 15 minutes and 1 hour, preferably about 30 minutes. For the glycine terminal peptides on the benzhydrylamine resins, the resin cleavage and deprotection steps may be combined in a single step utilizing liquid hydrogen fluoride and anisole as described above. The fully deprotected polypeptide is then purified by a sequence of chromatographic steps employing any or all of the following types: ion exchange on a weakly basic resin in the acetate form; hydrophobic adsorption chromatography on underivatized polystyrene-divinylbenzene (for example Amberlite XAD); silica gel adsorption chromatography; ion exchange chromatography on carboxymethylcellulose; partition chromatography, e.g. on Sephadex G-25, or countercurrent distribution; high performance liquid chromatography (HPLC), especially reverse phase HPLC on octyl- or octadecylsilyl-silica bonded phase column packing.

If a racemic amino acid is used in the 6-position, the diastereomeric nonapeptide or decapeptide final products are separated, and the desired peptide containing a D-amino acid in the 6-position is isolated and purified, preferably during the above-described chromatographic process.

The preparation of peptides having C-terminal azaglycine amides is preferably done using classical peptide solution synthesis using known peptide intermediates. This is described in more detail in Example 3.

Thus, in another aspect the present invention relates to a method for preparing compounds of the formula

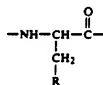


and the pharmaceutically acceptable salts thereof wherein:

V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue



wherein R is

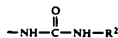
(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or

(b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydro-naphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycineamide or ---NH---R^1 , wherein

R^1 is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R^2 is hydrogen or lower alkyl, which process comprises:

(i) removing protecting groups and, optionally, covalently bound solid support from a protected polypeptide to afford a compound of Formula (I) or a salt thereof, and optionally

(ii) converting a compound of Formula (I) to a pharmaceutically acceptable salt, or

(iii) converting a salt of a compound of Formula (I) to a pharmaceutically acceptable salt, or

(iv) decomposing a salt of a compound of Formula (I) to a free polypeptide of Formula (I).

The following examples are given to enable those skilled in the art to more fully understand the practice the present invention. They should not be construed as a limitation upon the scope of the invention, but merely as being illustrative and representative thereof.

PREPARATION A

To an oven dried flask containing 0.1 L. of absolute ethanol (distilled from magnesium ethoxide) was added 1.52 g. of sodium metal. When hydrogen evolution ceased, 10.21 g. of ethyl 2-acetamido-2-cyanoacetate and 13.26 g. of 2-bromomethylnaphthalene were added to the solution. The solution was heated at reflux for 1 hour and then cooled. The ethanol was removed under reduced pressure and the residue was taken up in ethyl acetate. The organic layer was washed with two 50 mL. portions of water, one 50 mL. portion of saturated sodium chloride solution, and was dried over magnesium sulfate. The solution was filtered, the solvent was stripped off at reduced pressure and the residue was hydrolyzed in 100 mL. of concentrated hydrochloric acid at reflux for 2 hours.

The hydrolysis mixture was cooled and the precipitate of crude product was filtered. The crude product was redissolved in 0.5 L. of hot water containing 5 mL. of concentrated hydrochloric acid treated with charcoal, and the pH of the solution was adjusted to 6 with concentrated ammonium hydroxide. The precipitate was filtered and dried in vacuo to yield 11.3 g. of pure 3-(2-naphthyl)-D,L-alanine of melting point 230° - 232° C.

Repeating the above procedure, substituting a stoichiometrically equivalent amount of

1-bromomethylnaphthalene,

55 5-bromomethylnaphthalene,

9-bromomethylfluorene,

2-bromomethylfluorene,

2-bromomethylnaphthalene,

1-bromomethylphenanthrene,

60 α -chloroisodurene,

4-bromomethylbiphenyl,

1-bromomethyladamantane,

3-bromomethylphenanthrene

1-chloromethyl-2,4,6-tri-(n-butyl)benzene, and

1-chloromethyl-2,3,4,5,6-pentamethylbenzene,

for 2-bromomethylnaphthalene there are obtained the following amino acids:

3-(1-naphthyl)-D,L-alanine, m.p. 185° - 187° C.,

3-(9-anthryl)-D,L-alanine, m.p. 290° C. (HCl salt),
 3-(9-fluorenyl)-D,L-alanine,
 3-(2-fluorenyl)-D,L-alanine, m.p. 264°–269° C.,
 3-(2-anthryl)-D,L-alanine,
 3-(1-anthryl)-D,L-alanine,
 3-(2,4,6-trimethylphenyl)-D,L-alanine, m.p. 235°–237° C.,
 3-(4-biphenyl)-D,L-alanine, m.p. 290° C.,
 3-(1-adamantyl)-D,L-alanine,
 3-(3-phenanthryl)-D,L-alanine,
 3-(2,4,6-tri(n-butyl)phenyl)-D,L-alanine and
 3-(2,3,4,5,6-pentamethylphenyl)-D,L-alanine, respec-
 tively.

PREPARATION B

A solution of 18.2 g. 1,1-diphenylethylene, 25.3 g. methyl α -methoxy-N-benzyloxycarbonylglycinate, and 1.5 g. 2-naphthalenesulfonic acid in 300 mL. dry benzene was refluxed for 2 days. The crude product was purified on a column of silicic acid using a gradient of CH_2Cl_2 to $\text{CH}_2\text{Cl}_2/\text{EtOAc}$ (18:1). The purified methyl 2-[(2,2-diphenylethyl)phenyl]-N-benzyloxycarbonylglycinate was hydrolyzed to the corresponding acid with a solution of 10.9 g KOH in 350 mL. of 10% aqueous methanol. The resultant crude acid was dissolved in 100 mL. of 95% ethanol containing 3 mL. of conc. HCl and hydrogenated in the presence of 2 g. of 10% Pd on carbon for 24 hours to yield 2.4 g. of 3-(2,2-diphenylmethyl)-D,L-alanine, m.p. 235°–237° C.

PREPARATION C

To a solution of 12.9 g. of 3-(2-naphthyl)-D,L-alanine in 120 mL. of 1 M NaOH was added 6.23 mL. of acetic anhydride and 60 mL. of 1 M NaOH during 1 hour at 0° C. The pH was adjusted to 2 with conc. HCl and the resultant precipitate was filtered. The solid was recrystallized from 60% aqueous ethanol to yield 12.2 g. of N-acetyl-3-(2-naphthyl)-D,L-alanine.

To a solution of 15 g. of this N-acetyl amino acid in 240 mL. of dry methanol was added 15.8 mL. of boron trifluoride etherate and the mixture was refluxed for 1 hour. The alcohol was evaporated, 200 mL. water was added and the solution was extracted with ethyl acetate. The organic layer was washed with aqueous base and acid, dried over MgSO_4 , filtered, and stripped to an oil. Crystallization of this oil from ethyl acetate/hexane gave 14.2 g. of methyl N-acetyl-3-(2-naphthyl)-D,L-alaninate, m.p. 79°–80° C.

Repeating the above procedure, substituting a stoichiometrically equivalent amount of
 3-(1-naphthyl)-D,L-alanine,
 3-(2-fluorenyl)-D,L-alanine,
 3-(2-anthryl)-D,L-alanine,
 3-(1-anthryl)-D,L-alanine, and
 3-(2,2-diphenylmethyl)-D,L-alanine
 for 3-(2-naphthyl)-D,L-alanine there are obtained methyl N-acetyl-3-(1-naphthyl)-D,L-alaninate, m.p. 97.5°–98° C.,
 methyl N-acetyl-3-(2-fluorenyl)-D,L-alaninate, m.p. 170°–171° C.,
 methyl N-acetyl-3-(2-anthryl)-D,L-alaninate, and methyl N-acetyl-3-(2,2-diphenylmethyl)-D,L-alaninate, m.p. 113°–114° C., respectively.

PREPARATION D

A solution of 6.6 g. of methyl N-acetyl-3-(2-naphthyl)-D,L-alaninate in a mixture of 300 mL. of dimethyl-

sulfoxide, 120 mL. of 1 M KCl and 780 mL. of H_2O was treated with 33.6 mg. of the enzyme subtilisin in 3 mL. of 0.1 M KCl. The pH was maintained at 7 by means of automatic titration with 0.2 M NaOH by a Radiometer pH stat. After 30 minutes 70 mL. of NaOH solution had been taken up and the hydrolysis was stopped. The solution was made basic with 12 g. NaHCO_3 and was extracted with ethyl acetate. The organic layer contained methyl N-acetyl-3-(2-naphthyl)-D,L-alaninate. Crystallization from ethyl acetate/hexane gave a yellow solid, m.p. 80°–81° C.

This was converted to the free amino acid and then to the N-Boc amino acid as follows:

A solution of 2.5 g of methyl N-acetyl-3-(2-naphthyl)-D-alaninate in 60 ml of 6 N HCl was heated at 120°–130° for 3 hours and cooled to room temperature. The white precipitate which formed was collected and recrystallized from 50 ml of H_2O containing 1 ml of 12 N HCl by neutralization with NH_4OH to pH 6, and dried in vacuo to yield 1.2 g of 3-(2-naphthyl)-D-alanine, m.p. 242°–244°, $[\alpha]_D^{25}$ 26.6° (c 0.5, $\text{CH}_3\text{CO}_2\text{H}$).

A stirred solution of 3-(2-naphthyl)-D-alanine in a mixture of 55 ml of 1 N NaOH, 10 ml H_2O , and 20 ml dioxane was treated with 1.48 g of di-tert-butyl dicarbonate and 0.22 g of magnesium oxide at 0°. After 1.5 hours an additional 0.3 g of di-tert-butyl dicarbonate was added and the mixture was allowed to come to room temperature. The solid was removed by filtration and the filtrate was concentrated to 50 ml. This aqueous solution was brought to pH 2.5 with NaHSO_4 and extracted with ethyl acetate. The organic layer was washed with 5% NaHSO_4 , water and saturated salt solution. The ethyl acetate solution was dried over magnesium sulfate, filtered and concentrated to an oil which was crystallized from ether/hexane to yield 1.3 g of N-Boc 3-(2-naphthyl)-D-alanine, m.p. 90°–91°, $[\alpha]_D^{25}$ –32.6° (c 0.8, MeOH).

Repeating the above procedure substituting a stoichiometrically equivalent amount of
 methyl N-acetyl-3-(1-naphthyl)-D,L-alaninate,
 methyl N-acetyl-3-(2-fluorenyl)-D,L-alaninate,
 methyl N-acetyl-3-(2-anthryl)-D,L-alaninate, and
 methyl N-acetyl-3-(2,2-diphenylmethyl)-D,L-alaninate
 for methyl N-acetyl-3-(2-naphthyl)-D,L-alanine there are obtained the following N^t-Boc amino acids, via the corresponding free amino acids:
 N-Boc-3-(1-naphthyl)-D-alanine, m.p. 92°–93° C., $[\alpha]_D^{25}$ 54.8° (c 0.5 MeOH),
 N-Boc-3-(2-fluorenyl)-D-alanine, m.p. 161°–163° C. (dec.),
 N-Boc-3-(2-anthryl)-D-alanine, and N-Boc-3-(2,2-diphenylmethyl)-D-alanine, m.p. 153°–154° C., respectively.

PREPARATION E

In a Parr hydrogenation bottle was placed 0.85 g. of 3-(2-naphthyl)-D-alanine, 100 mL. of 2 M hydrochloric acid, and 0.85 g. of Adam's catalyst (PtO_2). The solution was packed under 60 lb/in² of H_2 gas for 20 hours in a Parr hydrogenation apparatus. The mixture was heated to dissolve the white precipitate and was filtered through diatomaceous earth. Concentration of the solution at reduced pressure followed by lyophilization from water yielded 0.8 g. of 3-(2-perhydnaphthyl)-D-alanine as a white solid of mp 230°–232° C.

This material was dissolved in a mixture of 3.2 mL. 1 N-NaOH, 5 mL. water, and 15 mL. dioxane, and was treated with 0.14 g MgO and 0.85 g. di-tert-butylidicar-

bonate. After 1 hour at 0° C. and 2 hours at 25° C. the suspension was filtered, concentrated to dryness at reduced pressure, the residue dissolved in water, washed with diethyl ether, and acidified to pH 2 with NaHSO₄. The acidified aqueous layer was extracted three times with ethyl acetate and the extracts were combined, dried over MgSO₄, filtered, and concentrated to give 0.75 g. of N-Boc-3-(2-perhydronaphthyl)-D-alanine as white oil.

A 0.1 g. portion of this material was dissolved in 5 ml tetrahydrofuran and titrated at 0° C. with freshly prepared diazomethane until the bright yellow color persisted. The reaction was quenched with 1 ml acetic acid, the solvent was evaporated and the residue was partitioned between 75 ml. ethyl acetate and 75 ml. water. The organic layer was washed with 5% NaHCO₃, water, 5% NaHSO₄, water, saturated NaCl solution, and dried over MgSO₄. The solution was filtered, concentrated under reduced pressure, and loaded on a preparative thin layer chromatography plate (750 μ thick, silica gel, 20 \times 20 cm.). The plate was developed with dichloromethane/ethyl acetate (18/1) and the product band was removed. The silica gel from the product band was washed with dichloromethane/ethyl acetate (9/1) on a fritted glass funnel and the filtrate was concentrated to give 0.1 g. of methyl N-Boc-3-(2-perhydronaphthyl)-D-alaninate as a light yellow oil.

This material was obtained as a mixture of two isomers at the 2 position of the perhydronaphthalene nucleus. These diastereomeric compounds may be separated on a high performance liquid chromatography column (Lichrosorb silica gel 60, 5 micron) with ethyl acetate/hexane (1/9) as eluent and hydrolyzed to the free acid, N-Boc-3-(2-perhydronaphthyl)-D-alanine.

Repeating the above procedure substituting a stoichiometrically equivalent amount of

3-(1-naphthyl)-D-alanine,
3-(2,2-diphenylmethyl)-D-alanine,
3-(2,4,6-trimethylphenyl)-D,L-alanine,
3-(4-biphenyl)-D,L-alanine,
3-(2,4,6-tri(n-butyl)phenyl)-D,L-alanine, and
3-(2,3,4,5,6-pentamethylphenyl)-D,L-alanine,
for 3-(2-naphthyl)-D-alanine there are obtained the following N-Boc amino acids:
N-Boc-3-(1-perhydronaphthyl)-D-alanine,
N-Boc-3-(perhydro-2,2-diphenylmethyl)-D-alanine,
N-Boc-3-(2,4,6-trimethylcyclohexyl)-D,L-alanine,
N-Boc-3-(perhydro-4-biphenyl)-D,L-alanine,
N-Boc-3-(2,4,6-tri(n-butyl)cyclohexyl)-D,L-alanine,
and
N-Boc-3-(2,3,4,5,6-pentamethylcyclohexyl)-D,L-alanine, respectively.

EXAMPLE I

In the reaction vessel of a Beckman 990 Peptide Synthesizer was placed 0.8 g. (0.8 mmol.) of benzhydrylamino-polystyrene-divinylbenzene resin (Lab Systems, Inc.) as described by Rivaile, supra. Amino acids were added sequentially to this resin by means of a synthesis program, as follows:

Step 1	CH ₂ Cl ₂ wash	1 time	1.5 min
2	50% CF ₃ CO ₂ H/CH ₂ Cl ₂	1 time	1.5 min
3	50% CF ₃ CO ₂ H/CH ₂ Cl ₂	1 time	30 min
4	CH ₂ Cl ₂ wash	3 times	1.5 min
5	10% triethylamine/CH ₂ Cl ₂	2 times	1.5 min

-continued

6	CH ₂ Cl ₂ wash	3 times	1.5 min
7	N ^o -Boc-amino acid solution	1 time	add
8	N,N-dicyclohexylcarbodiimide solution	1 time	add
9	CH ₂ Cl ₂ rinse and hold coupling	1 time	coupling reaction 2 hr
10	CH ₂ Cl ₂ rinse add	1 time	1.5 min
11	CH ₂ Cl ₂ wash	3 times	1.5 min
12	ethanol wash	3 times	1.5 min
13	CH ₂ Cl ₂ wash	3 times	1.5 min

Steps 1-13 complete a coupling cycle for one amino acid and completeness of the reaction is checked by the ninhydrin method of E. Kaiser, et al., *Anal. Biochem.*, 34, 595 (1970).

The resin was coupled sequentially with a 2.5 molar excess of each protected amino acid and DCC. Thus, the resin was treated during successive coupling cycles with

0.433 g. Boc-Gly-OH,
0.432 g. Boc-Pro-OH,
0.857 g. Boc-Arg(Tosyl)-OH,
0.462 g. Boc-Leu-OH,
0.504 g. Boc-3-(2-naphthyl)-D-alanine and 0.272 g. 1-hydroxybenzotriazole,
0.724 g. N-Boc-O-2-bromobenzyloxycarbonyl-L-tyrosine,
0.59 g. Boc-Ser(Benzyl)-OH,
0.608 g. Boc-Trp-OH,
0.654 g. Boc-His(Tosyl)-OH, and
0.524 g. pyroglutamic acid.

The resin was removed from the reaction vessel, washed with CH₂Cl₂ and dried in vacuo to yield 2.0 g. of protected polypeptide resin.

The polypeptide product was simultaneously removed from the resin and completely deprotected by treatment with anhydrous liquid HF. A mixture of 2.0 g. of protected polypeptide resin and 2 mL. of anisole (scavenger) in a Kel-F reaction vessel was treated with 20 mL. of redistilled (from CoF₃) anhydrous liquid HF at 0° C. for 30 minutes. The HF was evaporated under vacuum and the residue of (pyro)-Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂, as its HF salt, was washed with ether. The residue was then extracted with glacial acetic acid. The acetic acid extract was lyophilized to yield 0.8 g. of crude material.

The crude polypeptide was loaded on a 4 \times 40 cm. Amberlite XAD-4 column (polystyrene-4% divinylbenzene copolymer) and eluted with a concave gradient from water (0.5 L.) to ethanol (1 L.). The tubes containing fractions from effluent volume 690 mL. to 1,470 mL. were pooled and stripped to dryness to yield 490 mg. of partially purified polypeptide.

A 150 mg. sample of the partially purified product was subjected to partition chromatography on a 3 \times 50 cm. column of Sephadex G-25 using the solvent system 1-butanol/toluene/acetic acid/water containing 1.5% pyridine in the ratios 10:15:12:18. The pure fractions were pooled on the basis of thin layer chromatography (silica gel; BuOH/H₂O/HOAc/EtOAc; 1:1:1:1) and HPLC (5 micron, reverse phase, octadecylsilyl packing; 40% 0.03 M NH₄OAc/60% acetonitrile). The desired product came off the column in fractions from effluent volume 1,000 mL. to 1,400 mL. (Rf 0.1). The pure fractions were pooled, stripped to dryness, taken up in H₂O, and lyophilized to yield 57 mg of pure pyro-glutamyl-

alkyl in place of Aza-Gly-NH₂ affords the corresponding peptide with an AzaGly-NH-lower alkyl terminus, e.g.
 (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-AzaGly-Et,
 (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-N-methyl-Leu-Arg-Pro-AzaGly-Et and
 (pyro)Glu-His-Trp-Ser-Tyr-3-(2,4,6-trimethylphenyl)-D-Ala-Leu-Arg-Pro-AzaGly-Et.

EXAMPLE 4

Repeating the procedure of Example 1 and utilizing either a D-amino acid or a D,L amino acid at position 6 (in the latter case, separating the diastereomeric peptides during chromatography), substituting the appropriate amino acids in the solid phase synthesis sequence, there may be obtained the following decapeptides which are isolated and characterized as their acetic acid addition salts:

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-N-methylleucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-phenylalanyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-3-(1-naphthyl)-L-alanyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-phenylalanyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-3-(1-pentafluorophenyl)-L-alanyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(1-naphthyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-anthryl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-fluorenyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(3-phenanthryl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(4-biphenyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,2-diphenylmethyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(1-adamantyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,4,6-trimethylphenyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-[2,4,6-tri-(n-butyl)phenyl]-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,3,4,5,6-pentamethylphenyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,4,6-trimethylcyclohexyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-[2,4,6-tri-(n-butyl)cyclohexyl]-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-1-naphthyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-2-naphthyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(4-perhydrobiphenyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-2,2-diphenylmethyl)-D-alanyl-leucyl-arginyl-prolyl-glycinamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-isoleucyl-arginyl-prolyl-glycinamide; and
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-norleucyl-arginyl-prolyl-glycinamide.

EXAMPLE 5

Repeating the procedure of Example 2 and utilizing either a D-amino acid or a D,L amino acid at position 6 (in the latter case, separating the diastereomeric peptides during chromatography), substituting the appropriate amino acids in the solid phase synthesis sequence, there may be obtained the following nonapeptides which are isolated and characterized as their acetic acid addition salts:

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(1-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide;
 pyro-glutamyl-histidyl-phenylalanyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide;
 pyro-glutamyl-histidyl-3-(1-naphthyl)-L-alanyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-phenylalanyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-3-(1-pentafluorophenyl)-L-alanyl-3-(2-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(1-anthryl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide;
 pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(1-adamantyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide;

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(3-phenanthryl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(4-biphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,2-diphenylmethyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(1-adamantyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,4,6-trimethylphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-[2,4,6-tri-(n-butyl)phenyl]-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,3,4,5,6-pentamethylphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,4,6-trimethylcyclohexyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-[2,4,6-tri-(n-butyl)cyclohexyl]-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-1-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-2-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(4-perhydrobiphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-2-diphenylmethyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide,

mide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-N-methylleucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-isoleucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-norleucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

EXAMPLE 6

A. A solution of 0.1 g of the hydrogen fluoride salt of (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂ (See Example 1) is dissolved in 50 mL of water and passed through a column of 30 g Dowex 3 anion exchange resin which had previously been equilibrated with acetic acid and washed with deionized water. The column is eluted with deionized water and the effluent is lyophilized to yield the corresponding acetic acid salt of (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂, [α]_D²⁵ -27.5° (c 0.9, HOAc).

Repeating the above, substituting other acids for acetic acid during the equilibration of the resin, there may be obtained, for example, the corresponding salts with hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, benzoic acid, and the like.

Similarly there may be prepared the acid addition salts of other compounds of Formula I.

B. In the case of salts of low water solubility, these may be prepared by precipitation from water utilizing the desired acid. For example:

Zinc tannate salt—a solution of 10 mg of (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂ acetic acid salt in 0.1 mL of water was treated with a solution of 8 mg of tannic acid in 0.08 mL of 0.25 M NaOH. A solution of 5 mg of ZnSO₄ heptahydrate in 0.1 mL of water was immediately added to the solution of the LH-RH analogue.

The resultant suspension was diluted with 1 mL water and the precipitate was centrifuged. A supernatant was decanted and the residue was washed twice with 1 mL portions of water by centrifugation of the precipitate and decantation of the supernatant. The precipitate was dried in vacuo to yield 15 mg of the mixed zinc tannate salt of the above named LH-RH analogue.

Pamoate salt—to a solution of 50 mg (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂ acetic acid salt in a mixture of 1.6 mL of ethanol and 0.1 mL of 0.25 M NaOH was added solution of 11 mg of pamoic acid in 0.3 mL of 0.25 M NaOH. The solvents were removed at reduced pressure and the residue was suspended in 2 mL of water, centrifuged, and the supernatant was decanted. The precipitate was washed with 1.5 mL H₂O, centrifuged, and the supernatant was decanted. The precipitate was dried in vacuo to yield 54 mg of the pamoate salt of the above named LH-RH analogue.

- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(3-phenanthryl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(4-biphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,2-diphenylmethyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(1-adamantyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,4,6-trimethylphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-[2,4,6-tri-(n-butyl)phenyl]-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,3,4,5,6-pentamethylphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2,4,6-trimethylcyclohexyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-[2,4,6-tri-(n-butyl)cyclohexyl]-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-1-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-2-naphthyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(4-perhydrobiphenyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(perhydro-2-diphenylmethyl)-D-alanyl-leucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-N-methylleucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-isoleucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,
- pyro-glutamyl-histidyl-tryptophyl-seryl-tyrosyl-3-(2-naphthyl)-D-alanyl-norleucyl-arginyl-proline as its ethylamide, n-butylamide, cyclopropylamide, cyclohexylamide, trifluoromethylamide, pentafluoroethylamide and 2,2,2-trifluoroethylamide,

EXAMPLE 6

A. A solution of 0.1 g of the hydrogen fluoride salt of (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂ (See Example 1) is dissolved in 50 mL of water and passed through a column of 50 g Dowex 3 anion exchange resin which had previously been equilibrated with acetic acid and washed with deionized water. The column is eluted with deionized water and the effluent is lyophilized to yield the corresponding acetic acid salt of (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂, [α]_D²⁵ = -27.5° (c 0.9, HOAc).

Repeating the above, substituting other acids for acetic acid during the equilibration of the resin, there may be obtained, for example, the corresponding salts with hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, nitric acid, benzoic acid, and the like.

Similarly there may be prepared the acid addition salts of other compounds of Formula I.

B. In the case of salts of low water solubility, these may be prepared by precipitation from water utilizing the desired acid. For example:

Zinc tannate salt—a solution of 10 mg of (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂ acetic acid salt in 0.1 mL of water was treated with a solution of 8 mg of tannic acid in 0.08 mL of 0.25 M NaOH. A solution of 5 mg of ZnSO₄ heptahydrate in 0.1 mL of water was immediately added to the solution of the LH-RH analogue.

The resultant suspension was diluted with 1 mL water and the precipitate was centrifuged. A supernatant was decanted and the residue was washed twice with 1 mL portions of water by centrifugation of the precipitate and decantation of the supernatant. The precipitate was dried in vacuo to yield 15 mg of the mixed zinc tannate salt of the above named LH-RH analogue.

Pamoate salt—to a solution of 50 mg (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-Ala-Leu-Arg-Pro-Gly-NH₂ acetic acid salt in a mixture of 1.6 mL of ethanol and 0.1 mL of 0.25 M NaOH was added solution of 11 mg of pamoic acid in 0.3 mL of 0.25 M NaOH. The solvents were removed at reduced pressure and the residue was suspended in 2 mL of water, centrifuged, and the supernatant was decanted. The precipitate was washed with 1.5 mL H₂O, centrifuged, and the supernatant was decanted. The precipitate was dried in vacuo to yield 54 mg of the pamoate salt of the above named LH-RH analogue.

LH-RH Analogue	250 mg
Dextrose	5 gm
Benzyl alcohol	0.9 gm
Water, purified q.s. ad	100 ml

Dissolve LH-RH analogue, dextrose, benzyl alcohol in purified water and q.s. to volume.

E. Formulation for Rectal Administration
Suppository Vehicle for Rectal Administration

LH-RH Analogue	500 µg
Witepsol H15	20.0 gm

The LH-RH analogue is combined with the molten Witepsol H15, mixed well and poured into 2 gm molds. We claim:

1. A compound of the formula

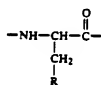


and the pharmaceutically acceptable salts thereof wherein:

V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue



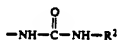
wherein R is

(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or

(b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycineamide or —NH—R¹, wherein R¹ is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R² is hydrogen or lower alkyl.

2. The compound of claim 1 wherein V is tryptophyl or phenylalanyl; W is tyrosyl; X is 3-(2-naphthyl)-D-alanyl or 3-(2,4,6-trimethylphenyl)-D-alanyl; Y is leucyl or N-methyl-leucyl; and Z is glycineamide or —NHEt.

3. The compound of claim 2 wherein X is 3-(2-naphthyl)-D-alanyl.

4. The compound of claim 2 which is (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂ and the pharmaceutically acceptable acid salts thereof.

5. The compound of claim 3 which is (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-N-methyl-Leu-Arg-Pro-Gly-NH₂ and the pharmaceutically acceptable salts thereof.

6. The compound of claim 3 which is (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-NHEt and the pharmaceutically acceptable salts thereof.

7. The compound of claim 3 which is (pyro)Glu-His-Trp-Ser-Tyr-3-(2-naphthyl)-D-alanyl-N-methyl-Leu-Arg-Pro-NHEt and the pharmaceutically acceptable salts thereof.

8. The compound of claim 3 which is (pyro)Glu-His-Phe-Ser-Syr-3-(2-naphthyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂ and the pharmaceutically acceptable salts thereof.

9. The compound of claim 2 wherein X is 3-(2,4,6-trimethylphenyl)-D-alanyl.

10. The compound of claim 9 which is (pyro)Glu-His-Trp-Ser-Tyr-3-(2,4,6-trimethylphenyl)-D-alanyl-Leu-Arg-Pro-Gly-NH₂ and the pharmaceutically acceptable salts thereof.

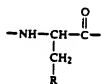
11. A method of inhibiting ovulation in a female mammalian subject which method comprises administering to said subject an effective amount of a compound of the formula



30 or a pharmaceutically acceptable salt thereof wherein: V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

35 X is a D-amino acid residue



wherein R is

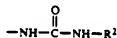
(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or

(b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycineamide or —NH—R¹, wherein

R¹ is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R² is hydrogen or lower alkyl, or a pharmaceutical composition containing same.

12. A pharmaceutical composition for inhibition of ovulation and treating endometriosis in a female mammalian subject and treating benign prostatic hyperplasia and inhibiting spermatogenesis in a male mamma-

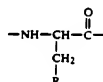
lian subject comprising an effective amount of a compound of the formula



or a pharmaceutically acceptable salt thereof wherein:
V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue



wherein R is

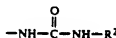
(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or

(b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycineamide or —NH—R¹, wherein

R¹ is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R² is hydrogen or lower alkyl, in admixture with a pharmaceutically acceptable non-toxic carrier.

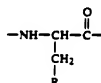
13. A method of treating endometriosis in a female mammalian subject which method comprises administering to said subject an effective amount of a compound of the formula



or a pharmaceutically acceptable salt thereof wherein:
V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue



wherein R is

(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or

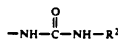
(b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted

with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycineamide or —NH—R¹, wherein

R¹ is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R² is hydrogen or lower alkyl, or a pharmaceutical composition containing same.

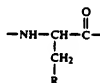
14. A method of treating benign prostatic hypertrophy in a male mammalian subject which method comprises administering to said subject an effective amount of a compound of the formula



or a pharmaceutically acceptable salt thereof wherein:
V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue



wherein R is

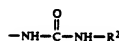
(a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or

(b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl;

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl;

Z is glycineamide or —NH—R¹, wherein

R¹ is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R² is hydrogen or lower alkyl, or a pharmaceutical composition containing same.

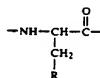
15. A method of inhibiting spermatogenesis in a male mammalian subject which method comprises administering to said subject an effective amount of a compound of the formula



or a pharmaceutically acceptable salt thereof wherein:
V is tryptophyl, phenylalanyl or 3-(1-naphthyl)-L-alanyl;

W is tyrosyl, phenylalanyl or 3-(1-pentafluorophenyl)-L-alanyl;

X is a D-amino acid residue

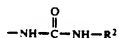


wherein R is

- (a) a carbocyclic aryl-containing radical selected from the group consisting of naphthyl, anthryl, fluorenyl, phenanthryl, biphenyl, benzhydryl and phenyl substituted with three or more straight chain lower alkyl groups; or
 (b) a saturated carbocyclic radical selected from the group consisting of cyclohexyl substituted

with three or more straight chain lower alkyl groups, perhydronaphthyl, perhydrobiphenyl, perhydro-2,2-diphenylmethyl and adamantyl,

Y is leucyl, isoleucyl, nor-leucyl or N-methyl-leucyl; Z is glycnamide or $-\text{NH}-\text{R}^1$, wherein R^1 is lower alkyl, cycloalkyl, fluoro lower alkyl or



wherein

R^2 is hydrogen or lower alkyl, or a pharmaceutical composition containing same.

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3641H-2

PATENTS
Case No. 21060

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In Re Application Of U.S. Patent No.
4,234,571

SERIAL NO. 47,661 : GROUP ART UNIT

FILED June 11, 1979 : EXAMINER

TITLE NONAPEPTIDE AND DECAPEPTIDE DERIVATIVES OF LUTEINIZING HORMONE
RELEASING HORMONE

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

TRANSMITTAL LETTER

Transmitted herewith for filing in the above-entitled patent
application is/are the following:

1. Application for Extension of Patent Term Under 35 U.S.C. 156 and
37 C.F.R. 1.710 et seq.

- [X] Please charge to deposit account No. 19-5430 the amount
of \$550.00.
- [X] The Commissioner is hereby authorized to charge any
additional fees or credit any overpayment to account No.
19-5430. A duplicate copy of this letter is enclosed.

Respectfully submitted,

Lester E. Johnson
Registration No. 28,023

Syntax Patent Law and Licensing Dept.
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Date: April 6, 1990

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